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14. ABSTRACT Many therapeutic strategies for SLE focus on the central role that autoantibody-producing B cells play in the pathology of this disorder. One general immunotherapeutic theme employs monoclonal antibodies (mAb) to interfere with, and/or deplete, B cells to ultimately reduce disease causing autoantibody levels. However, current strategies are inherently limited because they are not specific for the disease state. Thus, treatments that can specifically block autoantibody production without compromising B cell function are needed. In our application we presented preliminary evidence in an in vivo model of a related autoimmune disease (rheumatoid arthritis) that showed antibodies to RhoB, a small GTPase blocked autoantibody secretion without affecting the overall B cell repertoire. This data led us to propose the purpose of this study, to evaluate the ability of anti-RhoB antibodies to reduce levels of pathologic autoantibodies, ultimately attenuating the severity of symptoms in the MRL-lpr murine model of SLE. We have not yet completed testing the anti-RhoB therapy in the SLE model, but plan to do so by the end of the award period. Presently our data suggests that dosing with the anti-RhoB antibodies produces a trend towards a decrease in autoantibody titers and proteinuria; however, additional numbers are needed to achieve statistical significance. Over the remaining award period we will work to achieve statistical significance in our results.						
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INTRODUCTION:

Systemic lupus erythematosus (SLE) affects approximately 300,000 to over a million individuals in the United States with a female gender bias of 90%. Many therapeutic strategies for SLE focus on the central role that autoantibody-producing B cells play in the pathology of this disorder. One general immunotherapeutic theme employs monoclonal antibodies (mAb) to interfere with and/or deplete B cells to ultimately reduce disease causing autoantibody levels. However, current strategies are inherently limited because they are not specific for the disease state. Thus, treatments that can specifically block autoantibody production without compromising B cell function remain lacking. Our work aims to address this therapeutic gap. The objective of our study is to evaluate the ability of anti-RhoB peptide antibodies to reduce levels of pathologic autoantibodies, ultimately attenuating the severity of symptoms in the MRL-lpr murine model of SLE. Our research strategy includes the determination of autoantibody levels over the course of disease progression in animals treated with therapeutic and control antibodies. Additionally, autoantibody levels will be compared to renal pathology to correlate autoantibody levels with attenuation of disease symptoms. This study will lay the groundwork for developing an innovative therapeutic strategy to improve the care of SLE patients, address important and timely scientific questions, as well as, focus on a major unmet medical need.

BODY:

Objective to complete in the award period of 18 months.

Evaluate the ability of anti-RhoB antibodies to attenuate the severity of symptom in the MRL/MpJ-Faslpr (abbrev. MRL-lpr) animal model of SLE.

Task I. Prepare anti-RhoB antibody. *Months 1-3*

Task II. Obtain approval for animal study. *Months 1-3*

During the first 3 months of the award period the first two tasks listed in the SOW were completed. During this time we refined the methodology and began purification of the RhoB antibodies (7F7 and 9G5), as well as, completed the approval process for our animal studies. Unfortunately, during this time it was determined that we had a mite outbreak in the animal facility and this outbreak has complicated the completion of the remaining tasks.

Task III. Obtain MRL/MpJ-Faslpr female mice from approved vendor. *Month 3*

Task IV. Administer anti-RhoB antibody to MRL-*lpr* mice and monitor for the development of autoantibodies and proteinuria. Mice will be dosed regularly during the course of the experiment, approximately 6 months. We anticipate repeating the experiment to reach statistical significance. *Months 3-15*

Task V. Perform ELISA analyses to evaluate serum autoantibody levels. *Months 3-15*

Task VI. Final serum autoantibody levels will be determined, as well as the levels of cells actively secreting anti-chromatin antibodies by ELISpot from isolated spleens. Renal function will be determined by monitoring for proteinuria. Renal pathology will be scored for nephritis using histological kidney sections. *Months 10-16*

Task VII. Statistically analyze the data. *Months 10-18*

We are currently working to complete tasks 3 through 7. Because of the detection of mites in the animal facility we did not import mice until the treatment for the outbreak was completed. All mice in the facility were treated topically with a 5% moxadectin solution (August 2012). We waited to import mice and begin treatment until testing results indicated the mite outbreak was cleared (October 2012). However, approximately 3 weeks after we had imported and begun treating the first set of MRL-lpr mice with our targeted therapy, mites were again found in the facility. With this second outbreak all animals in the facility were fed ivermectin-containing food, and in our study, we continued to treat the

MRL-lpr mice with our RhoB-targeted therapy. Analysis of the autoantibody levels from this group of mice did not show differences between the groups. However, we are concerned about the significance of the result from this first set of animals because ivermectin treatment has been shown to affect immune responses [1-3]. Additionally, in two other mouse models of autoimmune disease that received the ivermectin containing food, changes were observed in altered disease development and antibody levels as compared to studies done prior to the detection of mites and subsequent ivermectin treatment.

In February 2013, we imported a second group of MRL-lpr mice after ivermectin treatment ended and sentinel screening was clear. The mice were divided into 3 treatment groups that received anti-RhoB antibody 7F7 or 9G5, or mouse IgG as a control. Dosing began at 4 weeks of age and continued one dose per week until the end of the experiment. We assessed the development of serum autoantibody titers and proteinuria. The data presented in Figure 1, panel A suggest a trend towards a decrease in titers for anti-double stranded DNA antibodies in the groups treated with the anti-RhoB antibodies compared to control IgG. Additionally, the data shown in Figure 1, panel B suggests a trending decrease in proteinuria with treatment of the anti-RhoB antibodies. None of these differences reached statistical significance. Kidney tissue from these mice was isolated and histological samples will be scored to assess renal pathology. The data presented in Figure 1 suggests that anti-RhoB antibodies may affect autoantibody titer and proteinuria in MRL-lpr mice; however, additional numbers are needed to achieve statistical significance.

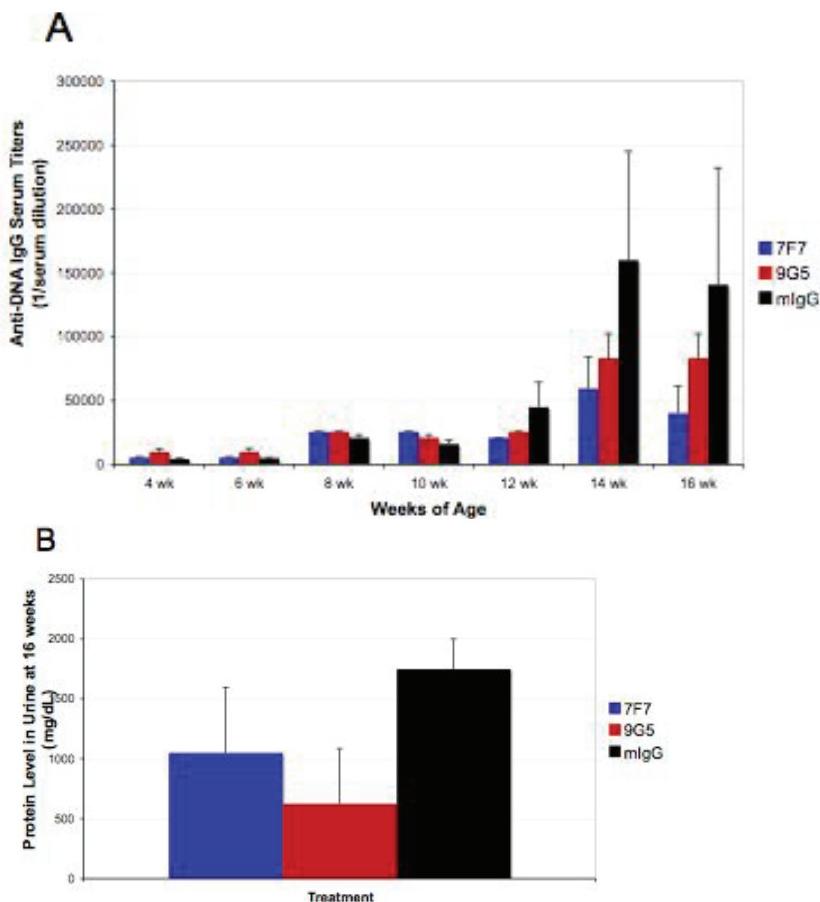


Figure 1: Autoantibody and proteinuria levels from MRL-lpr mice treated with anti-RhoB antibodies 7F7 or 9G5. Panel A: ELISA results for serum antibody titers to double-stranded DNA. Serum titers were determined at indicated ages during the treatment period. Anti-RhoB antibodies 7F7 and 9G5 were compared to control mouse IgG. Panel B: Proteinuria was determined at 16 weeks of age prior to euthanasia. N=4-5 mice per group.

During the remaining period of the award we will repeat our analysis using additional mice. We are in the process of adding animal numbers to our IACUC and DOD animal protocols. Once appropriate approvals have been received we will continue with our study.

KEY RESEARCH ACCOMPLISHMENTS:

There are no research accomplishments thus far from the study.

REPORTABLE OUTCOMES:

There are no reportable outcomes thus far from the study.

CONCLUSIONS:

At present we are not able to conclude that our therapy can affect autoantibody levels in the MRL-lpr model of SLE. We will continue to work to meet the objective of our study, to evaluate the ability of anti-RhoB peptide antibodies to reduce levels of pathologic autoantibodies, which we believe will attenuate the severity of symptoms in this animal model. Despite the setback of fur mites in the animal facility, we plan to meet our goal and obtain preliminary data necessary to advance an innovative therapeutic strategy into the clinic for treatment of SLE patients.

REFERENCES:

1. Blakley, B.R. and C.G. Rousseaux, *Effect of ivermectin on the immune response in mice*. Am J Vet Res, 1991. **52**(4): p. 593-5.
2. Yan, S., X. Ci, N. Chen, C. Chen, X. Li, X. Chu, J. Li, and X. Deng, *Anti-inflammatory effects of ivermectin in mouse model of allergic asthma*. Inflamm Res, 2011. **60**(6): p. 589-96.
3. Roble, G.S., W. Boteler, E. Riedel, and N.S. Lipman, *Total IgE as a serodiagnostic marker to aid murine fur mite detection*. J Am Assoc Lab Anim Sci, 2012. **51**(2): p. 199-208.

APPENDICES:

None

SUPPORTING DATA:

None